IONIZING RADIATIONS AND PARAMETERS MODIFYING RADIATION STERILIZATION

The ionizing radiations include alpha, beta, gamma and X-rays, high energy electrons, protons, and neutrons. These radiations are termed ionizing because the primary means of energy dissipation is the ejection of electrons from atoms of the material through which it passes. Gamma rays and X-rays are deeply penetrating, and have similar effects on cells. They are the principal ionizing radiations studied in microbial systems. Although ionizing radiation is ordinarily considered destructive, the background radiation in our environment may be necessary for normal life processes (90).

Aerobic and anaerobic bacterial spores resist higher doses of radiation than vegetative cells. Radiation resistance is not uniform within a cell population (144) or among strains of the same species (96, 101, 108). Differences in surface properties make it possible to separate radiation resistant and nonresistant cells in a culture (94). Inactivation is essentially logarithmic with increasing radiation doses (96), but occasional spores may survive extreme radiation doses (9 Mrad) although the progeny of such survivors do not have unusual radiation resistance (97). A computer program has been designed for mathematically studying

radiation effects on cell populations (89).

The chemical and physical environment prior to, during, and after irradiation can influence the radioresistance of an organism (95, 109, 110, 116, 127, 176). Thus the environment can afford protection (72, 106, 123, 130, 131, 137, 138, 139) or increase the sensitivity of cells to ionizing radiation (72, 92, 106, 136, 142).

The nutritional environment after irradiation (119, 131) is also critical for expression of radioresistance since the requirements for resistant cells to initiate growth may be different from the requirements of unirradiated cells. A cell may be injured by radiation, fail to grow in ordinary culture media, yet maintain viability. Stapleton (119) and Stapleton, Engel and Orce (50) showed that cultural conditions influence the resistance of Escherichia coli to physical and chemical agents. Resistant cells of strain B/r (CSH) require certain amino acids in the post-irradiation plating media for expression of resistance. Since any sterilization process depends on absence of growth in culture media as an indication that a reliable process has been applied, a large number of injured but non-multiplying organisms would still be significant unless it can be shown that the likelihood of a favorable extraterrestrial environment is remote.

Radiation Inactivation. The threshold sterilizing dose for 10^4 Clostridium botulinum spores in neutral phosphate buffer was determined by Anellis and Koch (108) to be 1.4 Mrad. About 15 of 102 strains had partial survival at this dose of gamma rays. The most resistant strains had D values of 0.317 to 0.336 Mrad. The sterilizing efficiency of gamma radiation is additive with increments of radiation (113).

Nickerson, Proctor and Goldblith (87) found 1.5-2.0 X 10^6 rep cathode rays were required to sterilize foods inoculated with 160-1800 Clostridium sporogenes spores per g.

Duggan, Anderson, and Elliker (106) reduced the viability of some radiation resistant vegetative bacteria by a factor of about 10^{-5} with 3.0 Mrad and by a factor of over 10^{-9} with 4.0 Mrad.

Grecz, Walker and Anellis (114) found that the substrate composition influenced the shape of the gamma radiation survival curve for Clostridium botulinum after 0.7 and 0.9 Mrad administered over the temperature range -196 to +90 C. Depending on the substrate and dose, radiation resistance appeared lowest at -20 to +40 C. Grecz and his associates (115) observed that radiation resistance of C. botulinum was greater at -196 C than at 0 C. Doses of 3.9 and 3.0 Mrad were required to inactivate 5 X 10^4 spores at -196 and 0 C respectively.

Roberts (117) reported that young encysting cells of Azotobacter vinelandii are sensitive to low radiation doses. Gamma and X-ray doses of 30-100 rad inactivated 10-30 percent of the cells. About 90 percent were inactivated by 6,000 rad. This organism and Escherichia coli K12 (lambda) (118) are potential biological indicators of low radiation doses.

Drake, Evans, and Niven (65) determined the radiation resistance of spores of several cultures isolated from irradiated canned ham. The LD_{99} values were: Bacillus licheniformis, 249,000 rad; Bacillus coagulans, 338,000 rad; Bacillus cereus, 513,000 rad. The radiation resistance of Streptococcus faecium strain HS-5 was as high as most bacterial spores.

Exposure of Clostridium roseum spores to 350 kiloroentgens was found by Wooley, Collier, and Clark (146) to prevent outgrowth of 90 percent of the irradiated spores.

Etchells et al. (97) found that 3 Mrep were required to sterilize small cucumbers. One to ten spores usually survived 1-2 Mrep. Other bacteria, molds and yeast had few survivors after exposure to 500,000 - 750,000 rep. Radiation resistance of cultures has been increased (101) by successive irradiations, but Webb and Clark (151) obtained a significant increase in sensitivity of Nocardia corallina coccoids by successive X-ray irradiation.

Temperature. Efforts to lower the dose required for radiation sterilization of foods have resulted in numerous papers concerned primarily with chemical sensitizers and temperature control. The chemical applications would be of questionable value in spacecraft sterilization since the necessary aqueous environment would not subject internal portions of spacecraft components to the chemical.

Graikoski (75) studied the interaction of temperature and gamma irradiation on spores of Clostridium botulinum and putrefactive anaerobe NCA 3679. These spores were slightly more resistant to radiation at -70 than at 4 C. Spore survival was greatest at irradiation temperatures just below the termal lethal threshold. Above this threshold, 85 C for Clostridium botulinum, spores were rapidly inactivated by radiation. Survivors increased in number as the irradiation temperature was raised above room temperature. Although radiation sensitized spores to heat, the thermal lethal threshold was not lowered.

Graikoski noted that irradiation should be conducted at the thermal lethal threshold of the most heat resistant organism in order to evaluate this combined method of sterilization. He concluded that the best procedure for sensitizing spores to heat would utilize heating prior to irradiation, during irradiation, and after irradiation at a lethal temperature. Heat and irradiation must be applied simultaneously to demonstrate the temperature effect.

Bellamy, Erickson and Lynch (98) irradiated spores over the range -178 to 110 C with 1.5 MeV electrons. The resistance of Bacillus pumilis spores irradiated anaerobically was nearly independent of temperature between -178 and +72 C, but decreased linearly at irradiation temperatures from 72 to 100 C. Bacillus megaterium was least resistant at 0 C. Aerobically irradiated Bacillus megaterium spores do not, however, display a minimum sensitivity over the irradiation temperature range -70 to +80 C. Dried spores are much more radiation resistant than spore suspensions at elevated temperatures.

Nickerson, Proctor and Goldblith (87) obtained greater radiation resistance in C. sporogenes irradiated in nitrogen, vacuum, frozen and in vacuum, and frozen, than at room temperature in air. Licciardello and Nickerson (72) required less radiation to kill 90 percent of Clostridium sporogenes PA 3679 spores at 20 C than at 66-68 C or -78 C.

Duggan, Anderson and Elliker (106) did not observe differences in resistance when several radiation resistant vegetative bacteria were irradiated at 0 and 20 C. Cells irradiated at 40 and 50 C were much more sensitive to radiation. Heating the cultures prior to irradiation lowered the radiation resistance.

Drake, Evans and Niven (65) irradiated survivors of a heat treatment that killed 90 percent of Streptococcus faecium strain HS-5 cells. The survivor curve had less initial lag, but generally paralleled that obtained with unheated cells. Suspensions initially irradiated to 90 percent lethality were slightly more heat sensitive than unirradiated cells. The initial lag phase was again eliminated. Sodium chloride in the suspending medium appeared to sensitize the cells to both heat and irradiation.

Shoesmith (134) demonstrated that viability and pathogenicity were inactivated exponentially at 100 C in Clostridium tetani spores, requiring 15 and 25 min for a tenfold decrease in viable count and pathogenicity. The same loss in viability by gamma rays required 2.6×10^5 rad, while 6.0×10^5 rad were necessary for 90 percent loss in pathogenicity.

Postirradiation heating has been found to enhance radiation lethality or to increase survival. Ehret et al. (111) obtained restoration of a considerable fraction of anaerobically irradiated Bacillus megaterium spores on exposure to higher temperatures after irradiation. Damage was not reversible, however, if anaerobically irradiated spores were exposed to oxygen before being heated. Tallentire and Powers (141) were able to restore Bacillus megaterium spores dried in air by postirradiation heating.

Vacuum-dried spores treated similarly were not restored. According to Tallentire and Davies (104), the probability of postirradiation oxygen-induced lethality for dry Bacillus subtilis spores is greatest at 37, 45, and 50 C.

Harris and Whitefield (145) examined the influences of postirradiation temperatures in the range 8-37 C on the viability of Escherichia coli finally incubated at 37 C. X-irradiated cells had greatest survival if they were held for 24 hr at 15 C before viability was determined at 37 C. Dharkar (132) observed that gamma irradiation sensitized organisms in orange juice to subsequent heat treatment. The sterilizing radiation dose was 8×10^5 rad, but half this dose sufficed if the juice was incubated for 15 min at 50 C following irradiation.

Morgan and Reed (125) showed that gamma irradiation increased the susceptibility of thermophilic anaerobe NCA 3814 to subsequent heating at 240 F (116 C). Prior heating did not increase the radiation sensitivity of Bacillus coagulans and B. stearothermophilus.

Licciardello (128) obtained significantly greater destruction of Salmonella typhimurium when radiation (20,000 - 125,000 rad) and heat were applied simultaneously than when they were applied consecutively. The lethal effect was greatest at irradiation temperatures above 110 F (43 C). In another report, Licciardello (186) noted that a complementary effect occurs only when irradiation

precedes heat treatment. Spores differ in the degree to which radiation sensitizes them to heat. Several species are sensitized to a lesser extent when they are irradiated in complex organic media than in buffer. When Salmonella typhimurium was heated simultaneously with irradiation, there were far fewer survivors than when heating followed irradiation.

According to Grecz, Walker and Anellis (179), 0.4 Mrad gamma rays significantly reduced Clostridium sporogenes PA 3679 spore resistance to conventional heat and to a greater degree to microwave heat. Radiation resistance was only slightly affected if either mode of heating preceded irradiation.

Hollaender (88) noted that cells are more sensitive to heat after X-rays, but reactivation of an Escherichia coli strain occurred if incubation was at 40 C. Infrared around 10,000 A given before X-irradiation will increase the production of mutations in irradiated Aspergillus terreus (88).

Licciardello (127) found that organic matter and oxygen must be absent for vitamin K_5 to sensitize Salmonella to gamma radiation. Moderate temperatures did not enhance radiosensitization by K_5.

Gaseous Atmosphere.

Oxygen. Oxygen sensitizes virtually all biological systems and many chemical systems to inactivation or alteration by ionizing

radiations. The enhanced response has been termed the 'oxygen effect'. Cells are generally more radioresistant in the absence of oxygen (anoxia). Many agents which modify radiation damage do not act per se, but rather by bringing about a change in the oxygen concentration in the vicinity of the irradiated cell. Nitrogen gas is inert, and is generally bubbled through the suspension to achieve anoxia.

Oxygen present during irradiation greatly enhances the lethal action of ionizing radiation toward dry microorganisms (93, 99, 100, 103, 111, 120, 141, 171, 187) and also lessens survival of organisms in suspension during irradiation (72, 88, 112, 135, 140, 170). Bachofer and Pottinger (91), however, discovered that oxygen protects T_1 bacteriophage against both irradiation and hydrogen peroxide. Neither oxygen, air, nor nitrogen influenced survival of organisms in irradiated orange juice (132), presumably because of the presence of protective substances. Oxygen continues to cause lethal damage if present after irradiation (99, 103, 104, 110). Tallentire and Davies (103) discovered that water prevents the latent damage attributed to oxygen.

Nitric Oxide. Nitric oxide is also active in modifying radiation injury. Organisms exposed to nitric oxide in advance of radiation (102) displayed enhanced radiosensitivity. Tallentire and Dickinson

(105) demonstrated that dry Bacillus subtilis spores irradiated in vacuum displayed a postirradiation oxygen effect. Nitric oxide admitted between exposures to oxygen stopped further development of the oxygen effect.

Powers, Kaleta and Webb (171) observed that dry spores of Bacillus megaterium were protected when nitric oxide was present during irradiation or in the first few hours after irradiation in nitrogen. Ehret et al. (111) also found nitric oxide protected anaerobically irradiated Bacillus megaterium spores. Thermorestoration of a considerable fraction of these spores occurred on exposure to higher temperatures after irradiation. On the other hand, damage was not reversible if anaerobically irradiated spores were exposed to oxygen before they were heated.

Howard-Flanders and Jockey (140) found nitric oxide and oxygen to be equivalent in doubling the radiosensitivity of Shigella sonnei during irradiation. Lynch and Howard-Flanders (142) established that pretreatment of this species with nitric oxide in anoxia, or with N-ethylmaleimide (NEM), a sulfhydryl scavenger, rendered the organism more sensitive to anoxic irradiation. Their data indicated that lethality was greatest when sulfhydryl groups were depleted by NEM and irradiation took place in oxygen.

Ultraviolet Radiation. Gol'dat and Alikhanyan (171) found that if spores of Streptomyces aureofaciens were exposed to X-rays after

ultraviolet treatment the number of survivors was considerably higher than expected considering the two irradiation acting separately. Small ultraviolet radiation doses were also reported to be protective to Saccharomyces cerevisiae according to Elkind and Sutton (153, 171). The protective action was demonstrated for yeast exposed to ultraviolet either before or after X-rays. It is evident that ultraviolet and ionizing radiation in combination should not be considered a satisfactory sterilization procedure.

Visible Light. Elkind and Sutton (153, 171) found that visible light reverses the ultraviolet protection of X-ray lethality as well as ultraviolet lethality. Savage, Howell and Clark (147) noted that Nocardia corallina and Staphylococcus aureus could be photoprotected against X-ray inactivation. Staphylococcus aureus was protected if incubated at 37 C but not at 29 C. The physiological state of the organism and the culture medium were shown to influence the degree of photoprotection. These results suggest that radiation experiments should be performed in the dark for maximal lethality. If possible, the irradiated objects should be maintained in the dark.

Water. Tallentire, Dickinson and Collett (110) determined that the lethal efficiency of radiation increases by about 35 percent in changing from the driest spore state to a condition of 100 percent relative humidity at room temperature. They considered water vapor

partial pressure a factor influencing the sterilization efficiency of a given dose of radiation. Very dry Aspergillus terreus spores are notably resistant to X-rays (88).

Davis, Silverman and Keller (120) and Silverman, Davis and Keller (187) found that spores of several species maintained in ultrahigh vacuum for several days were more sensitive to gamma radiation when irradiated in air than in vacuum. Desiccant-dried spores irradiated in air were more resistant than the ultradry spores exposed to air during irradiation and were as resistant as ultradry spores irradiated in vacuum.

Radiation and Spacecraft Sterilization. The radiations in space would not sterilize a spacecraft in flight because of penetration and dose limitations (169). Sterilizing doses of radiation have been reported (6, 7) to degrade spacecraft components to the same degree as dry heat.

There are several limitations on the application of radiation in spacecraft sterilization. Gamma ray intensity is not uniform throughout a radiation field because of the spatial orientation of the Cobalt-60 radiation source. A dosimetry diagram must be prepared of the space utilized for radiation exposure. The radiation intensity decreases with time since the half-life of Cobalt-60 is 5.3 years. These factors relate to the time required for the subject to receive the desired radiation dose. The estimated

dose can be obtained as an average based on the intensities in planes perpendicular to the radiation source. The dose is therefore not precisely determined, but is assumed uniform for a small homogeneous subject.

Hall and Bruch (22) noted that only heat and radiation achieve both surface and internal sterilization. Dry heat was considered the preferred agent. The order in which heat and radiation are applied influences microbial survival as does the atmosphere during and after irradiation. Bruch (168) had suggested that dry heat and radiation in combination may prove feasible for sterilizing components degraded by sterilizing doses of each agent.

The internal construction of all components to be irradiated must be known to the personnel responsible for operating the radiation facility. Metallic parts of the irradiated objects will absorb and scatter incident radiation. Metallic elements would shield material not in the direct path of the rays so that part of the object will not receive the desired dose. Since radiation effects are not subject to interference by interruptions in dose delivery (113), it may be practicable to rotate or turn the component or module to expose all areas likely to be shadowed in any orientation. The inhomogeneity of the radiation field would impose a limit on the size module to be treated.

Radiation effects in materials are not generalized, but are a function of their chemical composition. One cannot properly relate radiation data obtained with model systems from which organisms are easily retrieved for viability studies to the essentially insoluble matrix of an electronic component. Formation and fixation of free radicals important in killing microorganisms will depend on the material harboring the microbes. Component simulants would therefore be useful in heat studies, but of questionable value in a radiation program. A radiation dose known to sterilize a model system might not be adequate to sterilize an electronic device which by chance included a radiation protective chemical intended to serve another function.

The gaseous atmosphere in the radiation chamber can be expected to influence radiation-induced chemical reactions in the surface of impermeable plastics and resins such as would be found in spacecraft components. At appropriate doses, reactions would include decomposition, volatilization, or a transformation which can interfere with the insulating or protective function of the material (169). If air is present, ozone and oxides of nitrogen will be produced as a consequence of irradiation. These gases may then react with the item being irradiated.

The atmosphere would therefore be most significant insofar as material deterioration is concerned, and secondly as a dose

reducing factor. Should it be feasible to apply radiation and heat below the threshold levels for material damage, and it is known that oxygen is not deleterious, it would be desirable to specify an air or oxygen atmosphere to introduce the oxygen effect on the surface contamination present on the irradiated object.